



Docket No. 49447-2USPX
2413-PME4974-529

#9
APR

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:
Paul North Bateman)
Serial No.: 09/658,302) Group Art Unit: 1641
Filed: September 8, 2000) Examiner: K. Padmanabhan

RECEIVED

AUG 30 2001

TECH CENTER 1600/2900

For: SEPARATION AND DETECTION OF SPERMATOZOA

Commissioner for Patents
Attention: Box Missing Parts
Washington, D.C. 20231

CERTIFICATE OF MAILING
I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Attention: Box Missing Parts, Washington, D.C. 20231

on: August 23, 2001

Signature Jay L. Mowery

Dear Sir:

CLAIM OF PRIORITY UNDER 35 U.S.C. § 119

Under the provisions of 35 U.S.C. 119 Applicant hereby claims the priority of British Patent Office patent application no. UK 9817795.9 filed on August 14, 1998, which is mentioned in the declaration of the above-identified application. A certified copy of the priority document is filed herewith.

Respectfully submitted,

James F. Lea
Reg. No. 41,143

Date: August 23, 2001
Jenkens & Gilchrist, P.C.
1445 Ross Avenue, Suite 3200
Dallas, Texas 75202-2799
214/855-4756 (Direct)
214/855-4300 (Fax)

THIS PAGE BLANK (USPTO)



09/658,302



INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

CERTIFIED COPY OF PRIORITY DOCUMENT

RECEIVED
AUG 30 2001
TECH CENTER 1600/2900

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

accordance with the Patents (Companies Re-registration) Rules 1982, if a company named this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

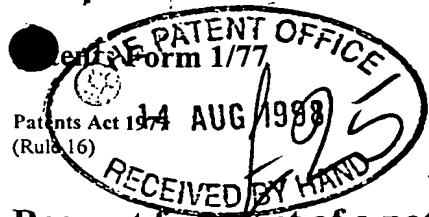
In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, L.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Dated 9 August 2001

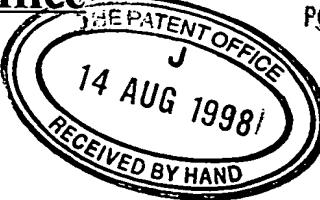
THIS PAGE BLANK (USPTO)



Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office



1 AUG 1998

1/77

17AUG98 E383545-1 D00019
P01/7700 25.00 - 9817795.9

The Patent Office
Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference

P19720GB

2. Patent application number

(The Patent Office will fill in this part)

9817795.9

3. Full name, address and postcode of the or of each applicant (*underline all surnames*)

GENOSIS, INC.
94 RIDGE STREET
WINCHESTER
MA 01890
USA

Patents ADP number (*if you know it*)

If the applicant is a corporate body, give the country/state of its incorporation

USA

74591241001

4. Title of the invention

A METHOD AND DEVICE FOR SEPARATION AND DETECTION OF SPERMATOZOA IN A SAMPLE

5. Name of your agent (*if you have one*)

"Address for service" in the United Kingdom to which all correspondence should be sent (*including the postcode*)

Carpmaels & Ransford
43 Bloomsbury Square
London
WC1A 2RA

Patents ADP number (*if you know it*)

83001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (*if you know it*) the or each application number

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
(*day / month / year*)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (*Answer 'Yes' if:*

- a) any applicant named in part 3 is not an inventor, or
- b) there is an inventor who is not named as an applicant, or
- c) any named applicant is a corporate body

Yes

See note (d))

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form.
Do not count copies of the same document

Continuation sheets of this form

Description	11
Claim(s)	6
Abstract	1
Drawing(s)	2 X ✓

-
10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (*Patents Form 7/77*)

Request for preliminary examination and search (*Patents Form 9/77*)

Request for substantive examination
(*Patents Form 10/77*)

Any other documents
(please specify)

11.

I/We request the grant of a patent on the basis of this application.

Signature  Date
14th August 1998

-
12. Name and daytime telephone number of person to contact in the United Kingdom

ADRIAN J. FISHER
Carpmaels & Ransford

0171 242 8692

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

- a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.
- b) Write your answers in capital letters using black ink or you may type them.
- c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.
- d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.
- e) Once you have filled in the form you must remember to sign and date it.
- f) For details of the fee and ways to pay please contact the Patent Office.

A method and device for separation and detection of spermatozoa in a sample.

The present invention relates to a method and kit for the separation and/or detection of motile spermatozoa in a sample, such a method and kit being useful in a number of applications, including the diagnosis and treatment of male infertility.

It has been estimated that approximately 14-16% of all couples attempting to get pregnant have difficulty in conceiving and are defined by fertility therapists as infertile. 40% of these cases result from male factors. In a substantial proportion of these, treatment is available to ameliorate or relieve the condition which leads to infertility.

Other conditions also exist in which it is desirable to test for the presence or otherwise of viable spermatozoa in a sample. For example, vasectomies are now frequently carried out as a method of contraception, but it is necessary to verify the effectiveness of a vasectomy by confirming that ejaculate is free of viable spermatozoa for a period of time after the operation.

A number of methods exist for assessing the motility and number of spermatozoa in a sample. One such method is microscopic analysis, which is typically carried out in a hospital or commercial laboratory. More recently, however, a number of proposals have been made for test kits which are intended to simplify the detection of spermatozoa, and which may therefore be useful in the diagnosis of male infertility. For example, WO97/40386 discloses a kit which is based on the detection of the 34kD human epididymal spermatozoa protein (P34H). This protein is thought to be involved in spermatozoa-zona pellucida interaction. The test kit disclosed in WO97/40386 uses an antibody raised against P34H or a related antigen, and a reagent for detecting antibody binding to P34H. As disclosed in WO97/40386, spermatozoa in a test sample are washed three times by centrifugation in Dulbecco-phosphate buffered saline. The samples are then heat denatured at 95°C, centrifuged at 14000 g, and the supernatants are then used for analysis.

EP-A-0387873 also discloses a kit for the evaluation of male fertility. This kit uses solid beads to which is bound an antibody specific to an antigenic site on the human

spermatozoon acrosome. Such beads are mixed with a test sample, and incubated for a period of 10 to 30 minutes. The test beads are then separated from the suspension, washed and subjected to measurement of the number of spermatozoa bound to the solid beads, preferably by examination with the aid of a microscope.

5

A kit for the detection of spermatozoa in a sample is also disclosed in WO95/29188. In this case, the test is based on antibodies to an antigen such as the SP-10 antigen of human spermatozoa.

- 10 A significant disadvantage of the test kits disclosed in the prior art mentioned above is that they do not distinguish between motile and non-motile spermatozoa. In the detection of male infertility, the ability to assess the numbers of motile spermatozoa is the most predictive indicator of male infertility. Moreover, many of the prior art test kits involve procedures, such as centrifugation or microscopic examination, which do
- 15 not lend themselves to home use, instead requiring implementation by a skilled practitioner.

- 20 It is therefore desired to provide a device, which can be self contained or provided in a plurality of components, and a method for separating motile spermatozoa from non-motile spermatozoa, and for collecting and detecting the presence of the motile spermatozoa.

- 25 According to a first aspect of the present invention there is provided an apparatus for separating motile spermatozoa from non-motile spermatozoa in a liquid sample, the apparatus comprising (i) a vessel having a sample receiving inlet, a filtered sample outlet and a sample separation filter mounted therebetween, the sample separation filter having a sample-receiving surface and an opposed surface, and the sample separation filter being effective substantially to prevent flow of the sample therethrough, but permitting passage of motile spermatozoa therethrough when said opposed surface of
- 30 said sample separation filter is placed in contact with a liquid medium and (ii) means for supplying a liquid to said opposed surface of said filter. The sample may comprise motile spermatozoa, non-motile spermatozoa and/or spermatozoa with reduced motility.

In order to detect the separated motile spermatozoa, spermatozoa detection means such as a spermatozoa detection filter may be provided at a sample outlet side of the sample separation filter, and spaced therefrom. The detection means may be integral with the apparatus, or it may be provided as a separate component thereof for inserting into the apparatus before, during or after placing the filter in contact with the liquid medium. The spermatozoa detection filter may have similar characteristics to the sample separation filter.

The filters may have a thickness of 100-2000 μm , preferably 200-1000 μm , and more 10 preferably 400-800 μm . For example, the filters may have a thickness of about 600 μm . The minimum particle retention size of the filters may be 5-100 μm , preferably 8-60 μm , and more preferably 10-40 μm . The filters may be fibrous, for example made of glass wool or polypropylene, or they may have a gel or a foam construction. For gel or foam constructions that are not self supporting, an underlying grid lattice or other support 15 may be provided. A particularly preferred filter is a glass fibre filter, which may include a binder such as an acrylic ester.

A reagent or a combination of reagents may be located in the spermatozoa detection means which are directly or indirectly capable of generating a visual signal on 20 interaction with spermatozoa. These reagent or combination of reagents may include antibodies that detect an antigen present on spermatozoa and/or may be capable of binding spermatozoa. Spermatozoa, when immobilised by such antibodies, could be visually detected using a visually detectable reagent which binds to spermatozoa. Antibodies to CD59, as discussed in GB9813112.1 and GB9813215.2, the complete 25 disclosures of which are incorporated herein by reference, have been found to be suitable for this purpose.

A spermatozoa chemoattractant, such as follicular fluid as identified in GB9813112.1 and GB9813215.2, may be located in the spermatozoa detection means. Such 30 spermatozoa chemoattractants are preferably located in a portion of the spermatozoa detection means distal from the sample separation filter.

A pick-up zone may be located either in the sample separation filter or the spermatozoa detection means, said pick-up zone comprising a reagent or combination of reagents which is/are capable of binding to spermatozoa and being transported therewith through the filter(s) to a detection area of the spermatozoa detection means. The reagent or
5 combination of reagents of the pick-up zone may include antibodies that detect an antigen present on spermatozoa. These antibodies may be detectably labelled, for example with gold particles.

The antibodies that are located in the detection area of the spermatozoa detection means
10 may recognise a different spermatozoa antigen compared to the antibodies located in the pick-up zone.

The spermatozoa detection means may comprise a spermatozoa acrosome-lysing reagent and a means for detecting pH change. The spermatozoa acrosome-lysing
15 reagent is typically a lysis buffer, and may comprise Proteinase K or the calcium ionophore A24297. The means for detecting pH change could be a pH sensitive probe or a pH indicator reagent capable of visually detecting a pH change, for example bromocresol purple.

20 The sample receiving surface of the sample separating filter may contain an enzymatic liquefaction agent , such as chymotrypsin, capable of causing semen liquefaction.

In a second aspect, the present invention provides a male fertility testing kit comprising an apparatus as described above, the kit further comprising a liquid release mechanism,
25 wherein upon activation of the liquid release mechanism, liquid from a liquid supply is applied to said opposed surface of the sample separation filter to provide liquid communication with a spermatozoa detection means. The spermatozoa detection means may be integral with the apparatus or it may be provided as a separate or separable component of the kit. The kit may comprise an integral liquid supply, or an external
30 liquid supply may be used. It will be appreciated that the liquid must be one in which motile spermatozoa remain motile for a sufficient period of time to migrate to the spermatozoa detection means, i.e. the liquid is generally non-toxic to spermatozoa. However, the liquid may be such that it has a toxicity to spermatozoa that is sufficiently

low that enough spermatozoa will successfully reach the detecting means. The liquid is preferably a buffer such as phosphate buffered saline (PBS) or Earle's Balanced Salt Solution (EBSS), as described in GB9813112.1 and GB9813215.2.

- 5 The kit may comprise two or more separable components, for example the apparatus and a base unit for the apparatus to engage with. Application of the apparatus to the base unit may be adapted to activate the liquid release mechanism, thereby wetting the spermatozoa detection filter and sample separation means. Alternatively, a button release for the liquid may be provided. The wetting of the spermatozoa detecting filter
- 10 may activate the detecting agents applied to the spermatozoa detecting means.

An overflow container for catching excess liquid applied to the apparatus upon activation of the liquid release mechanism may be provided.

- 15 The liquid supply may comprise a frangible compartment or portion, wherein the liquid release mechanism breaks the compartment to release liquid contained therein. The liquid thereby released may then be channelled by a ramp towards a well formed between the apparatus and a base portion of the kit. The compartment may be piercable by a liquid release mechanism in the form of a piercing mechanism, for example
- 20 retained by a stop, the stop preventing the piercing mechanism from piercing the compartment until activated by a user.

- According to a third aspect of the present invention, there is disclosed a method of detecting the presence of motile sperm in a sample, comprising the steps of providing a
- 25 filter having first and second surfaces, the filter permitting migration of the motile sperm therethrough when a liquid is applied to the second surface, applying the sample to the first surface, applying a liquid to the second surface, and detecting sperm that has migrated through the filter. The sample may be a mixture comprising motile and non-motile spermatozoa. The filter may be the filter contained within the apparatus or the
 - 30 kit as described above.

Preferred embodiments of a kit in accordance with the present invention and a method of use thereof will now be described by way of example with reference to the accompanying drawings in which:

- 5 Figure 1 shows a kit in accordance with the present invention prior to application of a vessel to a base unit; and

Figure 2 shows the kit of Figure 1, with an overflow, after application of the vessel to the base unit.

10

Figure 3 shows an alternative embodiment in which the detection means is a separable component of the kit.

A kit 10 for testing male fertility is shown in Figures 1 and 2. The kit comprises a
15 vessel 12, a base unit 14, a liquid supply 16 containing liquid 18, and two filters 20,22.

The first filter 20 is a sample separation filter 20 which forms a hindrance to transmission of spermatozoa due to the composition and construction thereof. For example, the filter will substantially retain on a sample receiving surface thereof
20 seminal fluid and non-motile spermatozoa present in a sample deposited thereon. This may be by virtue of the pore size of the filter, for example. Non-motile spermatozoa will not pass through the filter. However, where the spermatozoa are motile, they will be able to "swim" through the filter.

25 The head of a human spermatozoon is typically 3-5 μm in diameter, and tail length is approximately 50-60 μm . The filter should be such that these spermatozoa can swim through the filter upon application of the liquid to the opposed surface. Suitable filter materials may be identified by a series of simple experiments.

30 Experiment 1. The assessment of sperm toxicity.

A swim up from a semen sample is prepared (Practical Laboratory Andrology – David Mortimer, page 272) and the resultant preparation of motile spermatozoa is used for the

assessment of sperm toxicity of the filter. A 2cm by 2cm square of the filter is cut into 2mm by 1cm strips. 3x 1ml aliquots of the freshly prepared motile sample are added to round bottom tubes (e.g., Falcon no. 2001 or 2037) marked duplicate A, duplicate B and control C. 10 of the thin strips of filter are placed in each of duplicate A and B. A, B
5 and C are then incubated at 37°C for 1 hour with frequent agitation. Sperm motility and/or sperm vitality assessment is then performed (Practical Laboratory Andrology, pages 49-50 for motility and pages 66-69 for vitality) on both duplicates and the control. A marked difference in sperm motility and or vitality between the filter containing sample and the control indicates that the filter is toxic to sperm.

10

Experiment 2. The evaluation of sperm "wicking" through a filter.

200µl of liquefied semen is placed in a round bottom tube. A 0.5cm by 4cm strip of filter is then introduced to the semen sample, such that only the lower 1-2mm of the
15 filter is in direct contact with the semen sample. In some filters, the semen sample will move by capillary action or "wick" up the filter taking with it motile and non-motile spermatozoa, therein invalidating the separation. The extent of wicking in a given time frame e.g. 15 minutes, can be determined by removing the filter from the semen sample and analysing with light microscopy 2mm segments of the filter for the presence of
20 spermatozoa. In order to be an effective separator of motile spermatozoa from a mixture of motile and non-motile sperm, the extent of wicking in the filter (in the time frame that the sample will be left applied to the filter prior to detection or collection of the filtered motile sperm) should be less than the thickness of the filter.

25 Experiment 3. The effectiveness of a filter to prevent the passage of dead or immobile spermatozoa.

A sample of dead or immobilised sperm is obtained by either heating a semen sample at 95°C in a water bath or by adding a 10% cyanide solution. 200µl of a dead or
30 immobilised semen sample is applied to the upper surface of the filter, the underside of the filter being in direct communication with 1 ml of EBSS (Earles Balanced Salt Solution). After 5, 10, 15 and 30-minute intervals, a 10µl aliquot of the filtrate is removed and examined under light microscopy for the presence of spermatozoa. An

effective filter will not allow the passage of dead or immobile spermatozoa for the duration that the semen sample is required to be in contact with the upper surface of the filter.

5 Experiment 4. The effectiveness of a filter to selectively allow the passage of motile spermatozoa.

An assessment of the wet preparation of a semen sample is performed (Practical Laboratory Andrology, pages 49-50) and the characteristics noted. 250 μ l of the semen
10 sample is applied to the upper surface of the filter, the underside of the filter being in direct communication with 1 ml of EBSS (Earles Balanced Salt Solution). After 5, 10,
15 and 30-minute intervals, a 10 μ l aliquot of the filtrate is removed and examined under light microscopy for the presence of motile spermatozoa and the ratio of motile versus non-motile spermatozoa in the filtrate compared to that of the original sample. An
15 effective filter should allow the passage of motile spermatozoa. For example if the original semen sample had a motility of 40% (i.e. 60% of spermatozoa are non-motile), the filtrate should preferably have a motility of at least 90% with less than 10% being non-motile.

20 The second filter 22 of the kit is a spermatozoa detection filter 22. The spermatozoa detection filter 22 forms a detection zone 26 for the spermatozoa that can migrate through the sample separation filter 20. The detection zone 26 is provided within the spermatozoa detection filter 22, and comprises a reagent capable of generating a signal upon interaction with spermatozoa. However, a gap 24 is formed between the two
25 filters 20,22 to prevent activation of the kit until such time that a transport medium, for example the liquid 18 in the liquid supply 16, has been supplied to fill the gap 24 to enable the spermatozoa to be transmitted to the detection zone 26, and thus the fertility test to be conducted with the kit.

30 The composition and construction of suitable filters 20,22 and the detection zone 26 may be such as that described herein, or such as that described in further detail in the prior art. However, preferable compositions and constructions are as described in GB9813112.1 and GB9813215.2, the complete disclosures of which are incorporated

herein by reference. A particularly preferred filter material is identified as Filter 4622, available from Ahlstrom Filtration, Inc., 122 W. Butler Street, PO Box A, Mt. Holly Springs, PA 17065-0238, USA. It comprises a micro glass fibre with an acrylic binder. The acrylic latex is an anionic dispersion of acrylate polymers and copolymers in a water base. These polymers are based on acrylic esters. The pore size is 20 μm and the thickness is 580 μm .

The vessel 12 has a circular cross section with a side wall 28, an open top 30, an annular base 32 and an open nozzle 34 formed on the annular base 32. The spermatozoa detection filter 22 is provided within the nozzle 34. A cap (not shown) may be placed onto the open top 30 for maintaining a sterile environment within the vessel 12. The cap over the open top 30 would need to be removed for application of a sample into the vessel 12.

The base unit 14 comprises the liquid supply 16, a liquid release mechanism 36 and a well 38. The well 38 comprises a hole 40 adapted to receive the nozzle 34. The hole 40 may have a window (not shown) provided therein for inspection of the detection zone 26 after activation of the kit 10. The base unit 14 further comprises a ramp 42 for channelling liquid 18 from the liquid supply 16 into the well 38, and an overflow 44 for catching excess liquid 18 provided into the well 38 from the liquid supply 16.

The liquid release mechanism 36 is in the form of a piercing member 37 adapted to be activated by application of the vessel 12 onto the base unit 14. Prior to activation of the piercing member 37, the piercing member 37 is retained by a stop 48. Application of the vessel 12 draws the piercing member 37 past the stop 48. A frangible portion may be provided for the liquid supply 16 for puncturing with the piercing member 37 as it draws past the stop 48. This frangible portion may comprise a tin-foil wall section.

A process of performing a male fertility test with the above-described kit will now be described.

A sample of seminal fluid 46, or ejaculate, is deposited within an unused or recycled vessel 12 through the open top 30 and onto the sample separation filter 20. The sample

may be deposited, for example, by direct application by the user, or by a pipette application from an ejaculated semen sample. The thereby primed vessel 12 is then applied to an unused or recycled base unit 14 which has a fresh liquid supply 16 such that the nozzle 34 of the vessel 12 is inserted within the hole 40 of the base unit 14.

- 5 During the application of the vessel 12 to the base unit 14, the side wall 28 of the vessel 12 activates the piercing member 37 to puncture the liquid supply 16, as shown in figure 2, in which the piercing member 37 has pierced through the frangible portion of the liquid supply 16. Alternatively, a piercing button 50, see Figure 3, may be provided for piercing the liquid supply 16.

10

- Upon piercing the liquid supply 16, the liquid 18 contained within the liquid supply 16 is channelled down the ramp 42 into the well 38, filling the hole 40 and the well 38 with liquid 18, the liquid 18 also entering through the nozzle 34 of the vessel 12, which is located within the hole 40 of the base unit 14. Thereby, the liquid passes through the 15 spermatozoa detecting filter 22 to fill the gap 24 between the two filters 20,22 with the liquid 18. Excess liquid 18 overflows the well 38 and is collected by the overflow 44.

- The liquid 18 acts as a transport medium for spermatozoa that has migrated through the sample separation filter 20 to permit the spermatozoa to migrate beyond the sample 20 separation filter 20 towards the spermatozoa detecting filter 22, so that it may be detected by the detection zone 26 within the spermatozoa detecting filter 22. Upon detection of spermatozoa at the detection zone 26, a signal is produced by the reagent provided thereat, thus signifying presence of motile spermatozoa. In the absence of motile spermatozoa in the sample, no spermatozoa will reach the detection zone 26 25 since non-motile or reduced-motile spermatozoa will not pass through the sample separation filter 20.

- The kit 10 of Figure 3 is an alternative embodiment of the present invention. It has an open top 30 exposing a sample separation filter 20 as described above. A sample 46 is 30 shown deposited onto the sample separation filter 20 on a sample receiving surface thereof.

The side wall 28 of the kit 10 defines a well 38. A liquid supply 16 is provided integral with the kit 10, adjacent the well 38, the liquid supply 16 containing liquid 18 and having a frangible portion 60 on a periphery thereof for separating the liquid supply 18 from the well 38 prior to activation of the kit 10. A spermatozoa detection filter 22 is

5 provided on a slide member 56 which may be slidably attached to the kit 10, or it may be provided as a separate component of the kit insertable through a sealable opening 58 in the side wall 28 for conducting a detection of spermatozoa within the well 38.

The liquid release mechanism comprises a piercing button 50 and a piercing member

10 37. The piercing button 50 is biased into a non-piercing position by a spring 52.

Pressing the piercing button drives the piercing member 37 through the frangible portion 60 of the liquid supply, thus allowing liquid 18 contained in the liquid supply 16 to flow into the well 38.

15 Alternatively, the slide member 56 could be adapted to release the liquid 18 within the liquid supply 16 automatically upon insertion thereof within the well 38, for example by providing a piercing member thereon which would pierce the frangible portion 60 of the liquid supply 16.

20 A transparent window 54 is provided in the side wall 28 of the well 38 to enable an inspection of the detecting zone 26 contained within the spermatozoa detecting filter to be performed. However, the side wall 28 may be manufactured of a transparent material, thus avoiding the need for the window 54.

25 The principle of use for the kit 10 of Figure 3 is similar to that of the embodiments of Figures 1 and 2. However, the liquid 18 may be released into the well prior, during or after insertion of the spermatozoa detecting filter within the well.

It will, of course, be understood that the present invention, and in particular the kit and a
30 method of its use, has been described above purely by way of example. Modifications in detail may be made within the scope of the invention as defined in the claims.

Claims.

1. An apparatus for separating motile spermatozoa from non-motile spermatozoa in a liquid sample, the apparatus comprising (i) a vessel having a sample receiving inlet, a
5 filtered sample outlet and a sample separation filter mounted therebetween, the sample separation filter having a sample-receiving surface and an opposed surface, and the sample separation filter being effective substantially to prevent flow of the sample therethrough, but permitting passage of motile spermatozoa therethrough when said opposed surface of said sample separation filter is placed in contact with a liquid
10 medium and (ii) means for supplying a liquid to said opposed surface of said filter.
2. An apparatus according to claim 1, wherein the sample additionally comprises non-motile spermatozoa and spermatozoa with reduced motility.
3. An apparatus according to any preceding claim, comprising a spermatozoa detection means on the outlet side of the sample separation filter, and spaced therefrom.
- 15 4. An apparatus according to claim 3, wherein the detection means is integral with the apparatus.
5. An apparatus according to claim 3, wherein the detection means is a separable component of the apparatus for inserting into the apparatus before, during or after placing the sample separation filter in contact with the liquid medium.
- 20 6. An apparatus according to any preceding claim, wherein the filter has a thickness of 100-2000 μm .
7. An apparatus according to any preceding claim, wherein the filter has a thickness of 200-1000 μm , preferably 400-800 μm .
- 25 8. An apparatus according to any preceding claim, wherein the filter has a thickness off about 600 μm .
9. An apparatus according to any preceding claim,, wherein the filter has a minimum particle retention size of 5-100 μm , preferably 8-60 μm , more preferably 10-40 μm .

10. An apparatus according to any preceding claim, wherein the filter is fibrous.
11. An apparatus according to claim 10, wherein the fibrous filter is made of glass wool or polypropylene.
12. An apparatus according to any one of claims 6 to 9, wherein the filter is of a gel
5 or foam construction.
13. An apparatus according to any preceding claim, wherein the filter has an underlying grid lattice for supporting the filter.
14. An apparatus according to any of claims 3 to 13, wherein a reagent or a combination of reagents which is/are directly or indirectly capable of generating a
10 visual signal on interaction with spermatozoa is/are located in the spermatozoa detection means.
15. An apparatus according to claim 14, wherein the reagent or combination of reagents include antibodies that detect an antigen present on spermatozoa and are capable of binding spermatozoa.
- 15 16. An apparatus according to claim 15, wherein spermatozoa, when immobilised by the antibodies, can be visually detected using a visually detectable reagent which binds to spermatozoa.
17. An apparatus according to any of claims 3 to 16, wherein a spermatozoa chemoattractant is located in the spermatozoa detection means.
- 20 18. An apparatus according to claim 17, wherein the spermatozoa chemoattractant is located in a portion of the spermatozoa detection means distal from the sample separation filter.
19. An apparatus according to any of claims 3 to 18, wherein a pick-up zone is located either in the sample separation filter or the spermatozoa detection means, said
25 pick-up zone comprising a reagent or combination of reagents which is/are capable of binding to spermatozoa and being transported therewith to a detection area of the spermatozoa detection means.

20. An apparatus according to claim 19, wherein the reagent or combination of reagents of the pick-up zone include antibodies that detect an antigen present on spermatozoa.
21. An apparatus according to claim 20, wherein the antibodies that detect an antigen present on spermatozoa are detectably labelled.
5
22. An apparatus according to claim 21, wherein the antibodies that detect an antigen present on spermatozoa are detectably labelled with gold particles.
23. An apparatus according to claim 20, claim 21 or claim 22, wherein the antibodies that are located in a detection area of the spermatozoa detection means
10 recognise a different spermatozoa antigen compared to the antibodies located in the pick-up zone.
24. An apparatus according to claim 20, claim 21 or claim 22, wherein the antibodies that are located in a detection area of the spermatozoa detection means recognise the same spermatozoa antigen as the antibodies located in the pick-up zone.
- 15 25. An apparatus according to any of claims 3 to 24, wherein the spermatozoa detection means comprises a spermatozoa acrosome-lysing reagent and a means for detecting pH change.
26. An apparatus according to claim 25, wherein the spermatozoa acrosome-lysing reagent is a lysis buffer.
- 20 27. An apparatus according to claim 26, wherein the lysis buffer comprises Proteinase K or the calcium ionophore A24297.
28. An apparatus according to claim 25, claim 26 or claim 27, wherein the means for detecting pH change is a pH sensitive probe.
29. An apparatus according to claim 25, claim 26 or claim 27, wherein the means
25 for detecting pH change is a pH indicator reagent capable of visually detecting a pH change.

30. An apparatus according to claim 29, wherein the pH indicator reagent is bromocresol purple.
31. An apparatus according to any of the preceding claims, wherein the sample receiving surface of the sample separating filter contains an enzymatic liquefaction agent.
32. A male fertility testing kit comprising an apparatus according to any one of claims 3 to 31, the kit comprising a liquid release mechanism, wherein upon activation of the liquid release mechanism, liquid from a liquid supply is applied to the sample filtered end of the sample separation filter to provide liquid communication with the spermatozoa detection means.
33. A kit according to claim 32, wherein the detection means is a separable component of the kit.
34. The kit of claim 32 or claim 33, comprising an integral liquid supply.
35. The kit of claim 34, wherein liquid contained within the liquid supply is a buffer.
36. The kit of any one of claims 32 to 35, wherein the apparatus and a base unit form two separable components of the kit.
37. The kit of claim 36, wherein application of the apparatus to the base unit causes activation of the liquid release mechanism.
- 20 38. The kit of any one of claims 32 to 36, wherein a button release is provided for activating the liquid release mechanism.
39. The kit according to any of claims 32 to 38, wherein wetting the spermatozoa detection means with the liquid activates detecting agents applied thereto.
40. The kit according to any of claims 32 to 39, further comprising an overflow container for containing any excess liquid applied to the apparatus upon activation of the liquid release mechanism.

41. The kit according to any of claims 32 to 40 incorporating the liquid supply, the liquid supply comprising a frangible compartment, wherein the liquid release mechanism breaks the compartment to release liquid contained therein.
42. The kit according to claim 41, wherein liquid released from the compartment is channelled by a ramp towards a well formed between the apparatus and a base portion of the kit.
5
43. The kit according to claim 41 or claim 42, wherein the compartment is piercable and the liquid release mechanism is a piercing mechanism.
44. The kit according to claim 43, wherein the piercing mechanism is retained by a stop, the stop preventing the piercing mechanism from piercing the compartment until activated by a user.
10
45. A method of detecting the presence of motile sperm in a sample, comprising:
 - (a) providing a filter having first and second surfaces, the filter permitting migration of the motile sperm therethrough when a liquid is applied to the second surface,
 - 15 (b) applying the sample to the first surface,
 - (c) applying a liquid to the second surface, and
 - (d) detecting sperm that has migrated through the filter.
46. The method of claim 45, wherein the filter is the filter contained within the apparatus of any of claims 1 to 31.
- 20 47. The method of claim 43, wherein the filter is the filter contained within the kit of any of claims 32 to 44.
48. An apparatus for separating motile spermatozoa from non-motile spermatozoa substantially as hereinbefore described with reference to the accompanying drawings.
49. A male fertility testing kit substantially as hereinbefore described with reference 25 to the accompanying drawings.
50. A method for detecting an analyte in a sample substantially as hereinbefore described with reference to the accompanying drawings.

51. A method of detecting motile spermatozoa in a sample substantially as hereinbefore described with reference to the accompanying drawings.

ABSTRACT.

A method and device for separation and detection of spermatozoa in a sample.

- 5 A kit 10 for testing male fertility comprises a vessel 12, a base unit 14, a liquid supply 16 containing liquid 18, and two filters 20,22. The first filter 20 is a sample separation filter 20 which forms a hindrance to transmission of spermatozoa. The second filter 22 of the kit is a spermatozoa detection filter 22 comprising a reagent for identifying spermatozoa. Activation of the kit is prevented until a transport medium, such as the
- 10 liquid, fills a gap 24 allowing spermatozoa to transmit to a detection zone 26. The kit may be of one-piece construction and utilises a thin piece of filter material to separate motile from non-motile spermatozoa.

Figure 3 to accompany abstract

1/2

Fig. 1

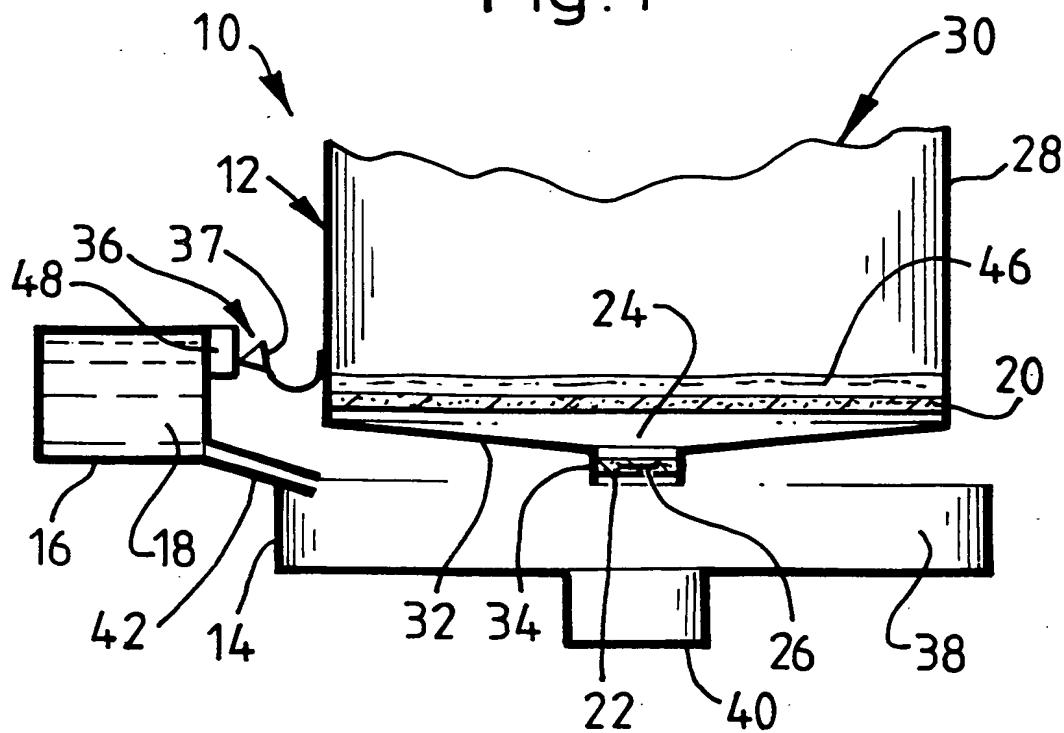
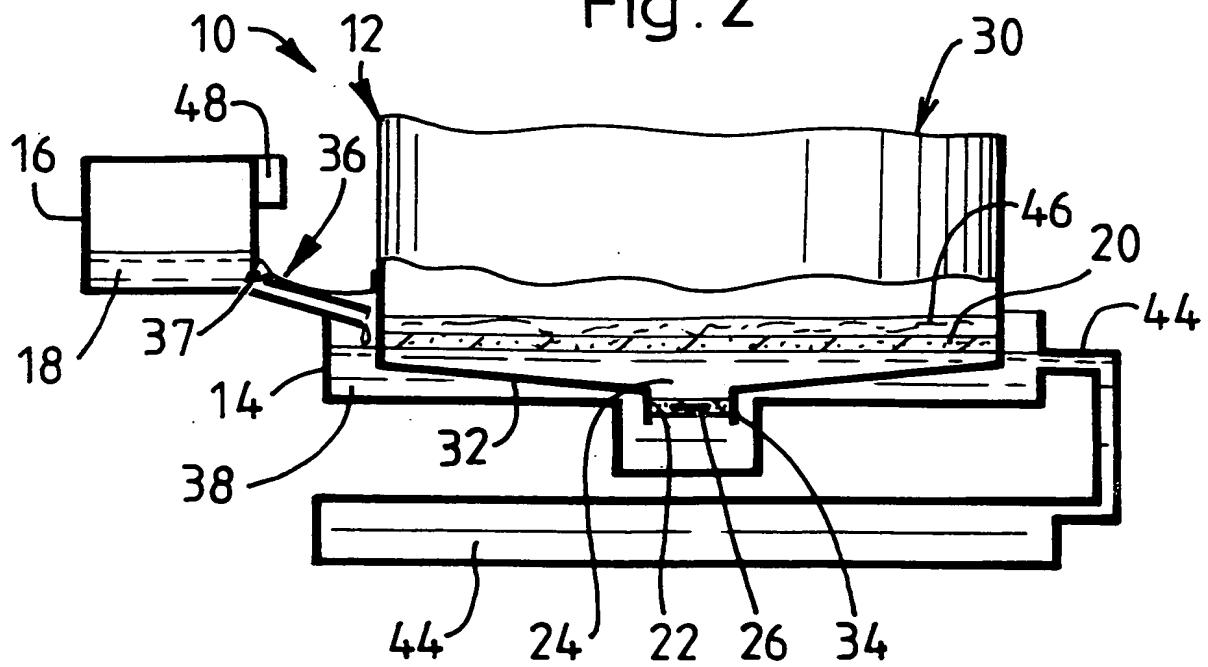
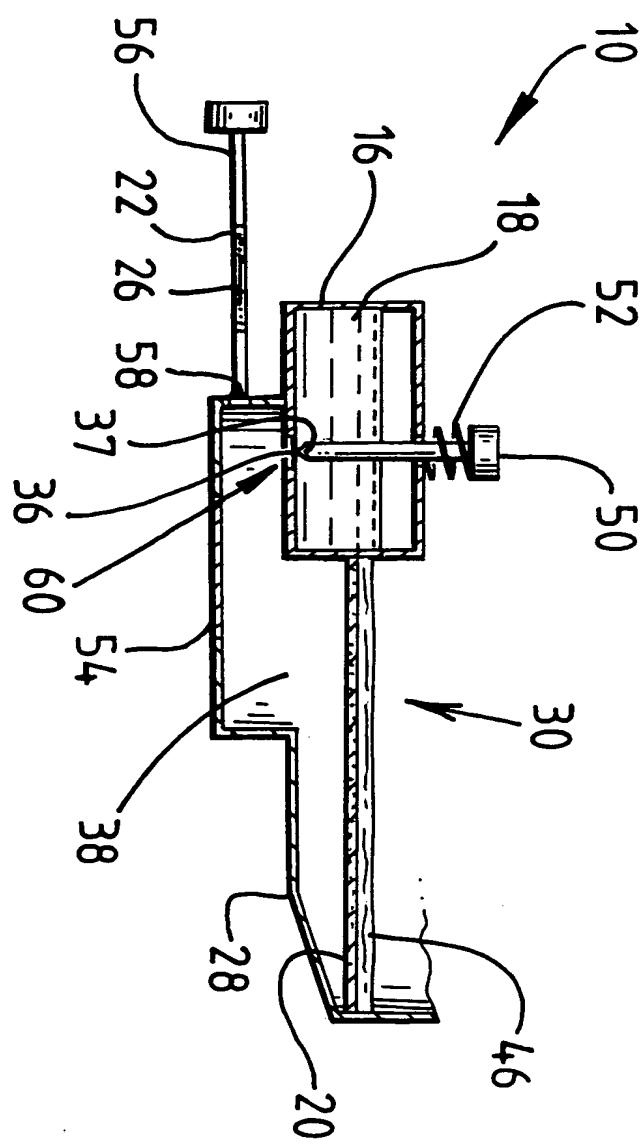


Fig. 2



THIS PAGE BLANK (USPTO)

Fig. 3



212

THIS PAGE BLANK (USPTO)